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Figure 1

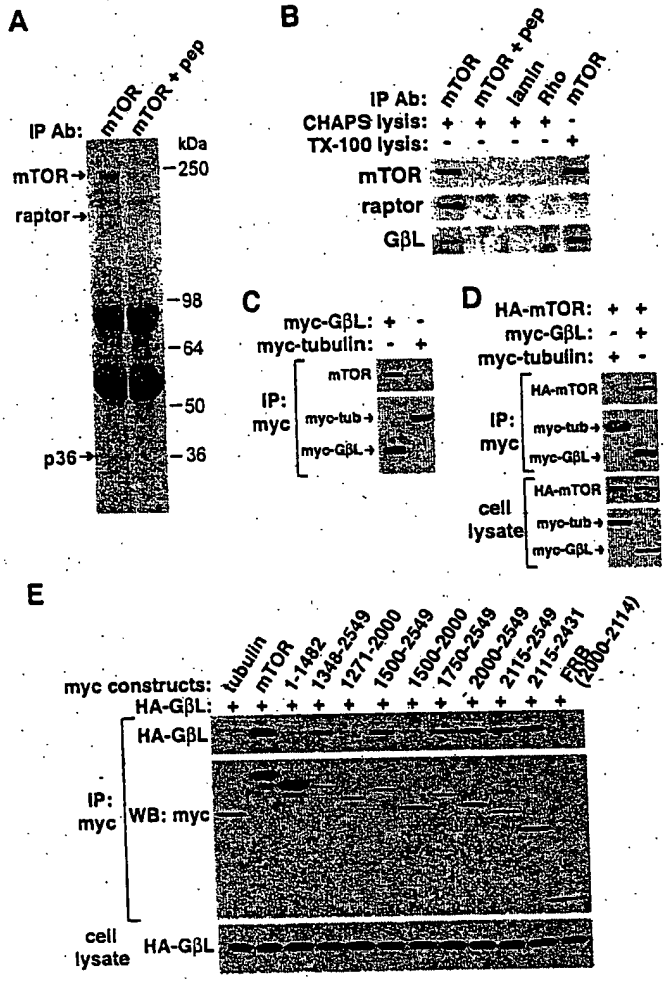
A
IP Ab:
mTOR
mTOR + pep
kDa
mTOR→
raptor→
p36→
250
98
64
50
36
B
IP Ab:
mTOR
mTOR + pep
lamin
Rho
mTOR
CHAPS lysis:
TX-100 lysis:
mTOR
raptor
GβL
C
myc-GβL:
myc-tubulin:
IP: myc
mTOR
myc-tub→
myc-GβL→
D
HA-mTOR:
myc-GβL:
myc-tubulin:
IP: myc
HA-mTOR
myc-tub→
myc-GβL→
cell lysate
HA-mTOR
myc-tub→
myc-GβL→
E
myc constructs:
tubulin
mTOR
1-1482
1348-2549
1271-2000
1500-2549
1500-2000
1750-2549
2000-2549
2115-2549
2115-2431
FRB (2000-2114)
HA-GβL:
HA-GβL
IP: myc
WB: myc
cell lysate
HA-GβL

Figure 2

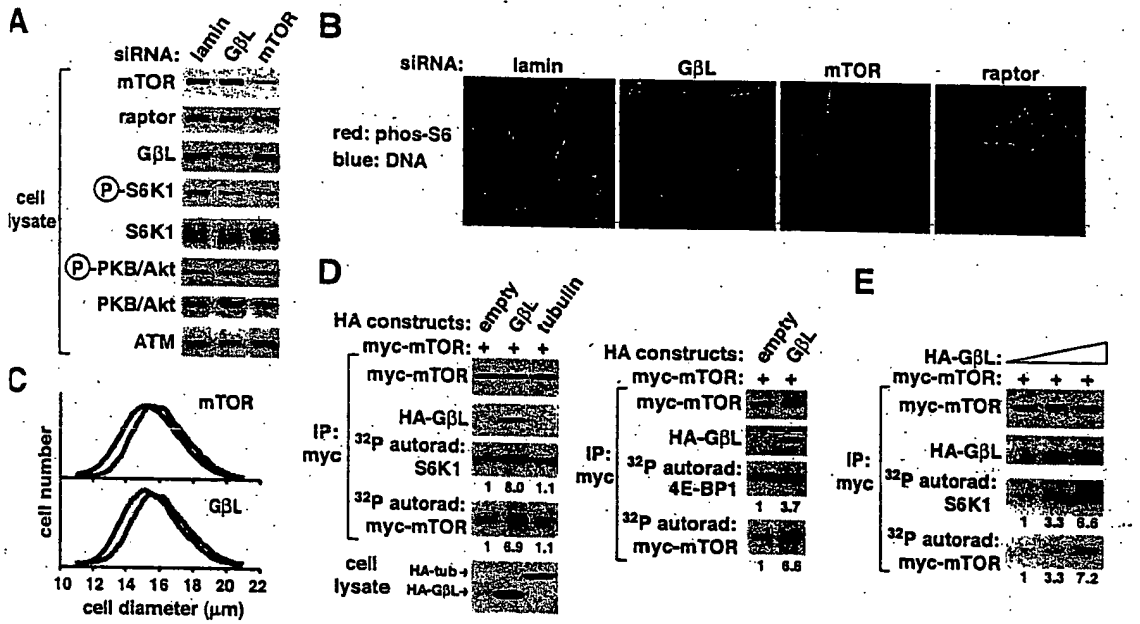
A
siRNA: lamin GβL mTOR
mTOR
raptor
GβL
Ⓟ-S6K1
S6K1
Ⓟ-PKB/Akt
PKB/Akt
ATM
cell lysate
B
siRNA: lamin GβL mTOR raptor
red: phos-S6
blue: DNA
C
mTOR
GβL
cell number
10 12 14 16 18 20 22
cell diameter (μm)
D
HA constructs: empty GβL tubulin
myc-mTOR: + + +
myc-mTOR
HA-GβL
IP: myc
32P autorad: S6K1
1 8.0 1.1
32P autorad: myc-mTOR
1 6.9 1.1
cell lysate
HA-tub→
HA-GβL→
HA constructs: empty GβL
myc-mTOR: + +
myc-mTOR
HA-GβL
IP: myc
32P autorad: 4E-BP1
1 3.7
32P autorad: myc-mTOR
1 6.6
E
HA-GβL:
myc-mTOR: + + +
myc-mTOR
HA-GβL
IP: myc
32P autorad: S6K1
1 3.3 6.6
32P autorad: myc-mTOR
1 3.3 7.2

Figure 3

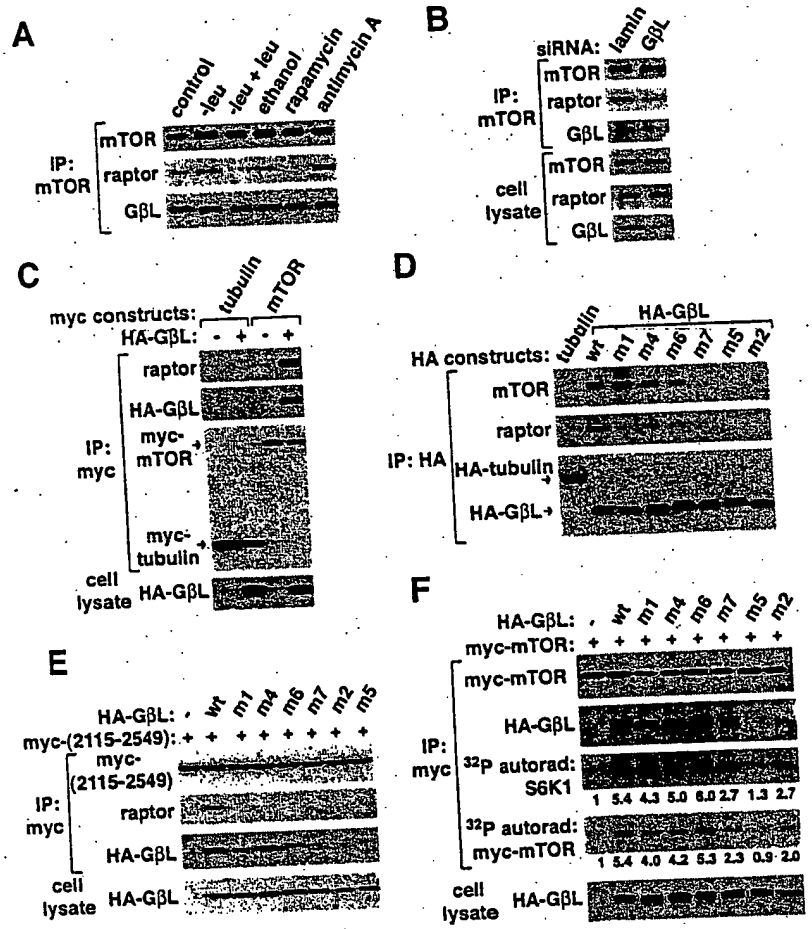
A
control
-leu
-leu + leu
ethanol
rapamycin
antimycin A
IP: mTOR
mTOR
raptor
GβL
B
siRNA: lamin
GβL
IP: mTOR
mTOR
raptor
GβL
cell lysate
mTOR
raptor
GβL
C
tubulin
mTOR
myc constructs:
HA-GβL: - + - +
raptor
HA-GβL
IP: myc
myc-mTOR
myc-tubulin
cell lysate
HA-GβL
D
tubulin
HA-GβL
wt m1 m4 m6 m7 m5 m2
HA constructs:
mTOR
raptor
IP: HA
HA-tubulin
HA-GβL
E
HA-GβL: wt m1 m4 m6 m7 m2 m5
myc-(2115-2549): + + + + + + +
myc-(2115-2549)
IP: myc
raptor
HA-GβL
cell lysate
HA-GβL
F
HA-GβL: wt m1 m4 m6 m7 m5 m2
myc-mTOR: + + + + + + +
myc-mTOR
HA-GβL
IP: myc
32P autorad: S6K1
1 5.4 4.3 5.0 6.0 2.7 1.3 2.7
32P autorad: myc-mTOR
1 5.4 4.0 4.2 5.3 2.3 0.9 2.0
cell lysate
HA-GβL

Figure 4

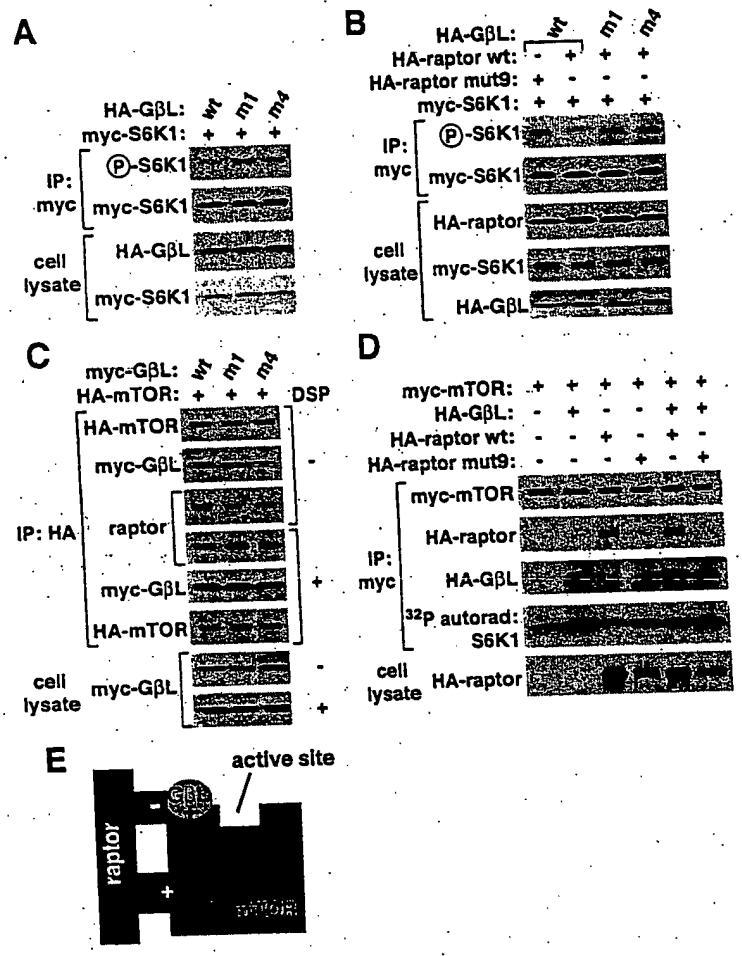
A
HA-GβL: wt m1 m4
myc-S6K1: + + +
IP: myc
Ⓟ-S6K1
myc-S6K1
cell lysate
HA-GβL
myc-S6K1
B
HA-GβL: wt m1 m4
HA-raptor wt: - + + +
HA-raptor mut9: + - - -
myc-S6K1: + + + +
IP: myc
Ⓟ-S6K1
myc-S6K1
cell lysate
HA-raptor
myc-S6K1
HA-GβL
C
myc-GβL: wt m1 m4
HA-mTOR: + + + DSP
IP: HA
HA-mTOR
myc-GβL
raptor
myc-GβL
HA-mTOR
-
+
cell lysate
myc-GβL
-
+
D
myc-mTOR: + + + + + +
HA-GβL: - + - - + +
HA-raptor wt: - - + - + -
HA-raptor mut9: - - - + - +
IP: myc
myc-mTOR
HA-raptor
HA-GβL
32P autorad: S6K1
cell lysate
HA-raptor
E
active site
GβL
raptor
-
+
mTOR

Figure 5a

p200 mRNA sequence (i.e. includes UTRs and ORF) CCTCTCTGAAGAACCTCCGAGTACGAGGGCG

AGCGGGTTGTGACTGAAACCCGTCAATATGGCGGCGATCGGCCGCGGCCGCTCTCTGAAGAACCTCCGAGTACGAGGGCG
GAATGACAGCGGCGAGGAGAACGTCCCGCTGGATCTGACCCGAGAACCTTCTGATAACTTAAGAGAGATTCTCCAAAATG
TGGCCAGATTGCAGGGAGTATCAAATATGAGAAAGCTAGGCCATCTGAATAACTTTACTAAGCTTCTTTGTGATATTGGC
CACAGTGAAGAAAAACTGGGCTTTCACTATGAGGATATCATAATTTGTTTGCGGTTAGCTTTATTAAATGAAGCAAAAGA
AGTGCGAGCAGCAGGGCTACGAGCGCTTCGATATCTCATCCAAGACTCCAGTATTCTCCAGAAGGTGCTAAAATTGAAAG
TGGACTATTTAATAGCTAGGTGCATTGACATACAACAGAGCAACGAGGTAGAGAGGACACAAGCACTTCGATTAGTCAGA
AAGATGATTACTGTGAATGCTTCCTTGTTTCCTAGTTCTGTGACCAACTCATTAATTGCAGTTGGAAATGATGGACTTCA
AGAAAGAGACAGAATGGTCCGAGCATGCATTGCCATTATCTGTGAACTAGCACTTCAGAATCCAGAGGTGGTGGCCCTTC
GAGGAGGACTAAACACCATATTGAAAAATGTGATTGATTGCCAATTAAGTCGAATAAATGAGGCCCTAATTACTACAATT
TTGCACCTTCTTAATCATCCAAAGACTCGGCAGTATGTGCGAGCTGATGTAGAATTAGAGSGAATTTTAGCACCCTATAC
TGATTTTCACTACAGACATAGTCCAGATACAGCTGAAGGACAGCTCAAAGAAGACAGAGAAGCACGATTTCTAGCCAGTA
AAATGGGAATCATAGCAACATTCCGATCATGGGCAGGTATTATTAATTTATGTAAACCTGGAAATTCTGGGATCCAGTCT
CTAATAGGAGTACTTTGCATACCAAATATGGAAATAAGGCGAGGTCTACTTGAAGTGCTTTATGATATATTTCGTCTTCC
TCTACCTGTTGTGACTGAGGAGTTCATAGAAGCACTACTCAGTGTAGATCCAGGGAGGTTCCAAGACAGTTGGAGGCTTT
CAGATGGCTTTGTGGCAGCTGAGGCAAAAACTATTCTTCCTCATCGTGCCAGATCCAGGCCAGACCTCATGGATAATTAT
TTGGCACTGATACTCTCTGCATTTATTCGTAATGGACTTTTAGAGGGTCTAGTCGAAGTGATAACAAACAGTGATGATCA
TATCTCAGTTAGAGCTACCATCCTTTTAGGAGAGCTTTTACATATGGCAAACACAATTCTTCCTCATTCACATAGCCATC
ATTTACACTGCTTGCCAACCCTAATGAATATGGCTGCATCCTTTGATATCCCCAAGGAAAAGAGACTGCGAGCCAGTGCA
GCCTTGAACTGTTTAAAACGCTTCCATGAAATGAAGAAACGAGGACCTAAGCCTTATAGTCTTCATTTAGACCACATTAT
TCAGAAAGCAATTGCAACACACCAGAAACGGGATCAGTATCTCCGAGTTCAGAAAGATATATTTATCCTTAAGGATACAG
AGGAAGCTCTTTTAATTAACCTTAGAGATAGCCAAGTCCTTCAACATAAAGAGAATCTTGAATGGAATTGGAATCTTATA
GGGACCATTCTTAAGTGGCCAAATGTAAATCTAAGAAACTATAAAGATGAACAGTTACACAGGTTTGTACGAAGACTACT
TTATTTTTACAAGCCCAGCAGTAAATTATATGCCAACCTGGATCTGGATTTTGCCAAGGCCAAACAGCTCACGGTTGTAG
GTTGCCAGTTTACAGAATTTCTTCTTGAATCTGAAGAGGATGGCAAGGCTACTTAGAAGATCTAGTAAAGGATATTGTT
CAGTGGCTCAATGCTTCATCTGGAATGAAACCCGAAAGAAGTCTTCAAAATAATGGTTTATTGACCACCCTTAGTCAACA
CTACTTTTTATTTATTGGAACACTTTCTTGCCACCCTCATGGAGTTAAAATGCTGGAAAAATGCAGTGTATTTCAGTGTC
TCCTTAATCTTTGCTCCTTGAAAAACCAAGATCACTTGCTAAAACTTACTGTTTCTAGCTTGGACTATAGCAGAGATGGA
TTGGCTAGAGTCATCCTTTCCAAAATTTTAACTGCAGCTACTGATGCCTGCAGACTCTATGCAACAAAACATTTAAGGGT

Figure 5b

ATTATTGAGAGCTAATGTTGAATTCTTTAATAATTGGGGAATTGAGTTGTTAGTGACCCAGCTACATGATAAAAACAAAA
CGATTTCCTCTGAAGCTCTTGATATCCTCGATGAAGCATGTGAAGACAAGGCCAATCTTCATGCTCTCATTCAGATGAAA
CCAGCGTTATCCCACCTTGGAGACAAGGGTTTGCTTCTCCTGCTGAGATTTCTCTCCATTCCAAAAGGATTTTCCTATCT
GAATGAAAGAGGTTATGTAGCAAAACAATTGGAAAAGTGGCACAGGGAATACAACTCCAAATATGTTGACTTGATTGAGG
AACAACTCAATGAAGCACTTACTACTTACCGGAAGCCTGTTGATGGTGATAACTATGTTCGTCGGAGTAACCAAAGATTA
CAGCGTCCTCACGTCTACCTGCCTATACACCTTTATGGACAACTAGTACACCATAAAACAGGCTGCCATTTGTTGGAAGT
ACAGAATATTATTACAGAACTCTGTCGTAATGTTCGTACACCAGATTTGGATAAGTGGGAAGAAATTAAAAAACTGAAAG
CATCTCTTTGGGCCTTGGGAAATATCGGCTCATCAAATTGGGGTCTCAATTTGCTACAGGAAGAAAACGTGATTCCAGAT
ATACTAAAACTTGCAAAACAGTGTGAAGTTCTTTCCATCAGAGGGACCTGTGTATATGTACTTGGGCTCATAGCTAAAAC
CAAACAAGGCTGTGATATTCTAAAATGTCACAACTGGGATGCTGTGAGGCATAGTCGCAAACATCTGTGGCCAGTGGTTC
CAGATGATGTGGAACAACTCTGTAATGAACTTTCATCTATCCCAAGCACTCTAAGTTTGAACTCGGAGTCAACCAGCTCT
AGACATAATAGTGAAAGTGAATCTGTGCCATCGAGTATGTTCATATTGGAGGATGACCGGTTTGGCAGCAGCTCTACTAG
TACATTTTTCCTTGATATCAATGAAGATACAGAGCCAACATTTTATGACCGATCTGGACCCATAAAGGATAAAAATTCAT
TCCCTTTCTTTGCTTCTAGTAAACTTGTGAAGAATCGTATCTTAAATTCGCTTACTTTGCCTAACAAAAAACATCGTAGT
AGCAGTGATCCAAAAGGAGGGAAATTATCATCTGAAAGTAAGACAAGCAACAGGCGAATCAGAACACTTACGGAGCCCAG
TGTTGATTTTAATCATAGTGATGATTTTACACCCATATCCACTGTACAGAAAACATTACAATTAGAGACTTCATTTATGG
GGAATAAGCACATTGAAGACACTGGTAGTACACCAAGCATTGGAGAAAATGACTTAAAATTCACCAAGAATTTTGGTACA
GAGAATCACAGAGAAAATACAAGCCGAGAGAGGTTAGTAGTAGAAAGTTCAACGAGCTCACATATGAAGATACGTAGCCA
AAGTTTCAATACAGACACTACAACAAGTGGCATAAGTTCAATGAGCTCAAGTCCTTCACGAGAGACAGTAGGTGTAGATG
CTACAACTATGGACACAGACTGTGGAAGCATGAGTACTGTGGTAAGTACTAAAACTATTAAGACAAGCCACTATTTGACG
CCACAGTCTAACCATCTGTCTCTCTCCAAATCAAATTCGGTGTCCCTGGTGCCTCCAGGTTCTTCTCATACGCTTCCTAG
AAGAGCACAGTCCCTTAAAGCACCCTCTATTGCTACAATTAAAAGTCTAGCAGATTGTAACTTTAGTTACACAAGTTCTA
GAGATGCTTTTGGCTATGCTACACTGAAAAGACTACAGCAACAAAGAATGCATCCATCCTTATCTCACTCTGAAGCTTTG
GCATCTCCAGCAAAAGATGTGCTATTTACTGATACCATCACCATGAAGGCCAACAGTTTTGAGTCCAGATTAACACCAAG
CAGGTTCATGAAAGCCTTAAGTTATGCATCATTAGATAAAGAAGATTTATTGAGTCCTATTAATCAAAATACCCTGCAAC
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GATATAAATGATATATTCCAGGTAAAGGATATTCCCTATTTTCAGACAAAAAACATACCACCACATGATGATCGAGGTGC
AAGAGCATTTGCCCATGATGCAGGAGGTCTTCCATCTGGAACTGGAGGTCTTGTAAAAAATTCTTTTCACTTGCTACGAC
AGCAGATGAGTCTTACGGAAATAATGAATTCAATCCATTCAGATGCCTCTCTGTTTTTAGAAAGTACAGAAGACACTGGA
CTACAGGAACATACAGATGATAACTGCCTTTATTGTGTCTGTATTGAAATTCTGGGTTTCCAGCCCAGCAACCAACTGAG
TGCAATATGTAGTCATTCAGACTTTCAAGATATTCCATATTCTGATTGGTGTGAGCAGACTATCCATAATCCTTTAGAAG
TGGTTCCCTCTAAGTTTTCGGGGATTTCTGGATGCAGTGATGGGGTGTCTCAAGAAGGCTCAGCTAGCAGCACCAAAAGC

Figure 5c

ACAGAATTGTTACTAGGTGTTAAAACAATTCCAGATGATACACCAATGTGCCGTATACTCCTTCGCAAAGAAGTTCTAAG
ATTAGTCATTAATTTGAGTAGTTCAGTTTCAACTAAATGTCATGAGACTGGGCTTTTAACAATTAAGGAGAAGTATCCTC
AAACATTTGATGACATATGCCTTTACTCTGAGGTTTCCCATTTGCTGTCACACTGCACATTCAGACTTCCGTGTCGGAGG
TTCATACAAGAATTATTTCAAGATGTACAGTTTCTACAAATGCATGAAGAAGCAGAGGCTGTGTTGGCAACACCACCAAA
GCAACCTATAGTTGATACATCTGCTGAATCCTGACCTCATATTTATGATGGATATAGATACATACTATATATATTCATAT
TTGTGGATTTCCTAAAAGCCTCAGAAAATACGACTGACTAGGCAGCAAAGACAGGAGTATCTTCTGTACACTGTTCCGCA
GTTACTGGTACATGAACAGTTGGAACTGCTGACTTTCCTAACCAAAACAACTTCCTTCTCTCCTTTGTTGAGCCTTTTGA
GGGGTTCATGATTCATTACCACAGTTTTAAGAGTTTCAGTTACCATTGTATGCAAGAGCCAAGCACTGAATACCTACATA
GGTTTTCTATTTTCTTTCATTTTAAAAGCGTAATGACAGTGGAACAATAATGGGATATGCAGAAGCACCCTTCACAAGTT
ATTTCTGAATGATTTTAGGGTAAATAATACAGATGCCTTGTATGTTAACTAACTTGTGGAAAGCAGGAATCAGTGTCTCT
AAGGCTGCATCCTATTACCACAATGGGGTTGTGCTATAACTGGCTGGTATTAGAGAGGGAAC

Figure 6a

p200 ORF sequence (i.e., no UTRs)

ATGGCGGCGATCGGCCGCGGCCGCTCTCTGAAGAACCTCCGAGTACGAGGGCGGAATGACAGCGGCGAGGAGAACGTCCC
GCTGGATCTGACCCGAGAACCTTCTGATAACTTAAGAGAGATTCTCCAAAATGTGGCCAGATTGCAGGGAGTATCAAATA
TGAGAAAGCTAGGCCATCTGAATAACTTTACTAAGCTTCTTTGTGATATTGGCCACAGTGAAGAAAAACTGGGCTTTCAC
TATGAGGATATCATAATTTGTTTGCGGTTAGCTTTATTAAATGAAGCAAAAGAAGTGCGAGCAGCAGGGCTACGAGCGCT
TCGATATCTCATCCAAGACTCCAGTATTCTCCAGAAGGTGCTAAAATTGAAAGTGGACTATTTAATAGCTAGGTGCATTG
ACATACAACAGAGCAACGAGGTAGAGAGGACACAAGCACTTCGATTAGTCAGAAAGATGATTACTGTGAATGCTTCCTTG
TTTCCTAGTTCTGTGACCAACTCATTAATTGCAGTTGGAAATGATGGACTTCAAGAAAGAGACAGAATGGTCCGAGCATG
CATTGCCATTATCTGTGAACTAGCACTTCAGAATCCAGAGGTGGTGGCCCTTCGAGGAGGACTAAACACCATATTGAAAA
ATGTGATTGATTGCCAATTAAGTCGAATAAATGAGGCCCTAATTACTACAATTTTGCACCTTCTTAATCATCCAAAGACT
CGGCAGTATGTGCGAGCTGATGTAGAATTAGAGSGAATTTTAGCACCCTATACTGATTTTCACTACAGACATAGTCCAGA
TACAGCTGAAGGACAGCTCAAAGAAGACAGAGAAGCACGATTTCTAGCCAGTAAAATGGGAATCATAGCAACATTCCGAT
CATGGGCAGGTATTATTAATTTATGTAAACCTGGAAATTCTGGGATCCAGTCTCTAATAGGAGTACTTTGCATACCAAAT
ATGGAAATAAGGCGAGGTCTACTTGAAGTGCTTTATGATATATTTCGTCTTCCTCTACCTGTTGTGACTGAGGAGTTCAT
AGAAGCACTACTCAGTGTAGATCCAGGGAGGTTCCAAGACAGTTGGAGGCTTTCAGATGGCTTTGTGGCAGCTGAGGCAA
AAACTATTCTTCCTCATCGTGCCAGATCCAGGCCAGACCTCATGGATAATTATTTGGCACTGATACTCTCTGCATTTATT
CGTAATGGACTTTTAGAGGGTCTAGTCGAAGTGATAACAAACAGTGATGATCATATCTCAGTTAGAGCTACCATCCTTTT
AGGAGAGCTTTTACATATGGCAAACACAATTCTTCCTCATTCACATAGCCATCATTTACACTGCTTGCCAACCCTAATGA
ATATGGCTGCATCCTTTGATATCCCCAAGGAAAAGAGACTGCGAGCCAGTGCAGCCTTGAACTGTTTAAAACGCTTCCAT
GAAATGAAGAAACGAGGACCTAAGCCTTATAGTCTTCATTTAGACCACATTATTCAGAAAGCAATTGCAACACACCAGAA

Figure 6b

ACGGGATCAGTATCTCCGAGTTCAGAAAGATATATTTATCCTTAAGGATACAGAGGAAGCTCTTTTAATTAACCTTAGAG
ATAGCCAAGTCCTTCAACATAAAGAGAATCTTGAATGGAATTGGAATCTTATAGGGACCATTCTTAAGTGGCCAAATGTA
AATCTAAGAAACTATAAAGATGAACAGTTACACAGGTTTGTACGAAGACTACTTTATTTTTACAAGCCCAGCAGTAAATT
ATATGCCAACCTGGATCTGGATTTTGCCAAGGCCAAACAGCTCACGGTTGTAGGTTGCCAGTTTACAGAATTTCTTCTTG
AATCTGAAGAGGATGGGCAAGGCTACTTAGAAGATCTAGTAAAGGATATTGTTCAGTGGCTCAATGCTTCATCTGGAATG
AAACCCGAAAGAAGTCTTCAAAATAATGGTTTATTGACCACCCTTAGTCAACACTACTTTTTATTTATTGGAACACTTTC
TTGCCACCCTCATGGAGTTAAAATGCTGGAAAAATGCAGTGTATTTCAGTGTCTCCTTAATCTTTGCTCCTTGAAAAACC
AAGATCACTTGCTAAAACTTACTGTTTCTAGCTTGGACTATAGCAGAGATGGATTGGCTAGAGTCATCCTTTCCAAAATT
TTAACTGCAGCTACTGATGCCTGCAGACTCTATGCAACAAAACATTTAAGGGTATTATTGAGAGCTAATGTTGAATTCTT
TAATAATTGGGGAATTGAGTTGTTAGTGACCCAGCTACATGATAAAAACAAAACGATTTCCTCTGAAGCTCTTGATATCC
TCGATGAAGCATGTGAAGACAAGGCCAATCTTCATGCTCTCATTCAGATGAAACCAGCGTTATCCCACCTTGGAGACAAG
GGTTTGCTTCTCCTGCTGAGATTTCTCTCCATTCCAAAAGGATTTTCCTATCTGAATGAAAGAGGTTATGTAGCAAAACA
ATTGGAAAAGTGGCACAGGGAATACAACTCCAAATATGTTGACTTGATTGAGGAACAACTCAATGAAGCACTTACTACTT
ACCGGAAGCCTGTTGATGGTGATAACTATGTTCGTCGGAGTAACCAAAGATTACAGCGTCCTCACGTCTACCTGCCTATA
CACCTTTATGGACAACTAGTACACCATAAAACAGGCTGCCATTTGTTGGAAGTACAGAATATTATTACAGAACTCTGTCG
TAATGTTCGTACACCAGATTTGGATAAGTGGGAAGAAATTAAAAAACTGAAAGCATCTCTTTGGGCCTTGGGAAATATCG
GCTCATCAAATTGGGGTCTCAATTTGCTACAGGAAGAAAACGTGATTCCAGATATACTAAAACTTGCAAAACAGTGTGAA
GTTCTTTCCATCAGAGGGACCTGTGTATATGTACTTGGGCTCATAGCTAAAACCAAACAAGGCTGTGATATTCTAAAATG
TCACAACTGGGATGCTGTGAGGCATAGTCGCAAACATCTGTGGCCAGTGGTTCCAGATGATGTGGAACAACTCTGTAATG
AACTTTCATCTATCCCAAGCACTCTAAGTTTGAACTCGGAGTCAACCAGCTCTAGACATAATAGTGAAAGTGAATCTGTG
CCATCGAGTATGTTCATATTGGAGGATGACCGGTTTGGCAGCAGCTCTACTAGTACATTTTTCCTTGATATCAATGAAGA
TACAGAGCCAACATTTTATGACCGATCTGGACCCATAAAGGATAAAAATTCATTCCCTTTCTTTGCTTCTAGTAAACTTG
TGAAGAATCGTATCTTAAATTCGCTTACTTTGCCTAACAAAAAACATCGTAGTAGCAGTGATCCAAAAGGAGGGAAATTA
TCATCTGAAAGTAAGACAAGCAACAGGCGAATCAGAACACTTACGGAGCCCAGTGTTGATTTTAATCATAGTGATGATTT
TACACCCATATCCACTGTACAGAAAACATTACAATTAGAGACTTCATTTATGGGGAATAAGCACATTGAAGACACTGGTA
GTACACCAAGCATTGGAGAAAATGACTTAAAATTCACCAAGAATTTTGGTACAGAGAATCACAGAGAAAATACAAGCCGA
GAGAGGTTAGTAGTAGAAAGTTCAACGAGCTCACATATGAAGATACGTAGCCAAAGTTTCAATACAGACACTACAACAAG
TGGCATAAGTTCAATGAGCTCAAGTCCTTCACGAGAGACAGTAGGTGTAGATGCTACAACTATGGACACAGACTGTGGAA
GCATGAGTACTGTGGTAAGTACTAAAACTATTAAGACAAGCCACTATTTGACGCCACAGTCTAACCATCTGTCTCTCTCC
AAATCAAATTCGGTGTCCCTGGTGCCTCCAGGTTCTTCTCATACGCTTCCTAGAAGAGCACAGTCCCTTAAAGCACCCTC
TATTGCTACAATTAAAAGTCTAGCAGATTGTAACTTTAGTTACACAAGTTCTAGAGATGCTTTTGGCTATGCTACACTGA
AAAGACTACAGCAACAAAGAATGCATCCATCCTTATCTCACTCTGAAGCTTTGGCATCTCCAGCAAAAGATGTGCTATTT

ACTGATACCATCACCATGAAGGCCAACAGTTTTGAGTCCAGATTAACACCAAGCAGGTTCATGAAAGCCTTAAGTTATGC
ATCATTAGATAAAGAAGATTTATTGAGTCCTATTAATCAAAATACCCTGCAACGATCTTCCTCAGTGCGGTCCATGGTGT
CCAGTGCCACATATGGGGGTTCAGATGATTACATTGGTCTTGCTCTCCCGGTGGATATAAATGATATATTCCAGGTAAAG
GATATTCCCTATTTTCAGACAAAAAACATACCACCACATGATGATCGAGGTGCAAGAGCATTTGCCCATGATGCAGGAGG
TCTTCCATCTGGAACTGGAGGTCTTGTAAAAAATTCTTTTCACTTGCTACGACAGCAGATGAGTCTTACGGAAATAATGA
ATTCAATCCATTCAGATGCCTCTCTGTTTTTAGAAAGTACAGAAGACACTGGACTACAGGAACATACAGATGATAACTGC
CTTTATTGTGTCTGTATTGAAATTCTGGGTTTCCAGCCCAGCAACCAACTGAGTGCAATATGTAGTCATTCAGACTTTCA
AGATATTCCATATTCTGATTGGTGTGAGCAGACTATCCATAATCCTTTAGAAGTGGTTCCCTCTAAGTTTTCGGGGATTT
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ATTCCAGATGATACACCAATGTGCCGTATACTCCTTCGCAAAGAAGTTCTAAGATTAGTCATTAATTTGAGTAGTTCAGT
TTCAACTAAATGTCATGAGACTGGGCTTTTAACAATTAAGGAGAAGTATCCTCAAACATTTGATGACATATGCCTTTACT
CTGAGGTTTCCCATTTGCTGTCACACTGCACATTCAGACTTCCGTGTCGGAGGTTCATACAAGAATTATTTCAAGATGTA
CAGTTTCTACAAATGCATGAAGAAGCAGAGGCTGTGTTGGCAACACCACCAAAGCAACCTATAGTTGATACATCTGCTGA
ATCCTGA

Figure 6c

Figure 7a

p200 amino acid sequence

MAAIGRGRSLKNLRVRGRNDSGEENVPLDLTREPSDNLREILQNVARLQGVSNMRKLGHLNNFTKLLCDIGHSEEKLGFH
YEDIIICLRLALLNEAKEVRAAGLRALRYLIQDSSILQKVLKLKVDYLIARCIDIQQSNEVERTQALRLVRKMITVNASL
FPSSVTNSLIAVGNDGLQERDRMVRACIAIICELALQNPEVVALRGGLNTILKNVIDCQLSRINEALITTILHLLNHPKT
RQYVRADVELEXILAPYTDFHYRHSPDTAEGQLKEDREARFLASKMGIIATFRSWAGIINLCKPGNSGIQSLIGVLCIPN
MEIRRGLLEVLYDIFRLPLPVVTEEFIEALLSVDPGRFQDSWRLSDGFVAAEAKTILPHRARSRPDLMDNYLALILSAFI
RNGLLEGLVEVITNSDDHISVRATILLGELLHMANTILPHSHSHHLHCLPTLMNMAASFDIPKEKRLRASAALNCLKRFH
EMKKRGPKPYSLHLDHIIQKAIATHQKRDQYLRVQKDIFILKDTEEALLINLRDSQVLQHKENLEWNWNLIGTILKWPNV
NLRNYKDEQLHRFVRRLLYFYKPSSKLYANLDLDFAKAKQLTVVGCQFTEFLLESEEDGQGYLEDLVKDIVQWLNASSGM
KPERSLQNNGLLTTLSQHYFLFIGTLSCHPHGVKMLEKCSVFQCLLNLCSLKNQDHLLKLTVSSLDYSRDGLARVILSKI
LTAATDACRLYATKHLRVLLRANVEFFNNWGIELLVTQLHDKNKTISSEALDILDEACEDKANLHALIQMKPALSHLGDK
GLLLLLRFLSIPKGFSYLNERGYVAKQLEKWHREYNSKYVDLIEEQLNEALTTYRKPVDGDNYVRRSNQRLQRPHVYLPI
HLYGQLVHHKTGCHLLEVQNIITELCRNVRTPDLDKWEEIKKLKASLWALGNIGSSNWGLNLLQEENVIPDILKLAKQCE
VLSIRGTCVYVLGLIAKTKQGCDILKCHNWDAVRHSRKHLWPVVPDDVEQLCNELSSIPSTLSLNSESTSSRHNSESESV
PSSMFILEDDRFGSSSTSTFFLDINEDTEPTFYDRSGPIKDKNSFPFFASSKLVKNRILNSLTLPNKKHRSSSDPKGGKL
SSESKTSNRRIRTLTEPSVDFNHSDDFTPISTVQKTLQLETSFMGNKHIEDTGSTPSIGENDLKFTKNFGTENHRENTSR
ERLVVESSTSSHMKIRSQSFNTDTTTSGISSMSSSPSRETVGVDATTMDTDCGSMSTVVSTKTIKTSHYLTPQSNHLSLS

Figure 7b

KSNSVSLVPPGSSHTLPRRAQSLKAPSIATIKSLADCNFSYTSSRDAFGYATLKRLQQQRMHPSLSHSEALASPAKDVLF
TDTITMKANSFESRLTPSRFMKALSYASLDKEDLLSPINQNTLQRSSSVRSMVSSATYGGSDDYIGLALPVDINDIFQVK
DIPYFQTKNIPPHDDRGARAFAHDAGGLPSGTGGLVKNSFHLLRQQMSLTEIMNSIHSDASLFLESTEDTGLQEHTDDNC
LYCVCIEILGFQPSNQLSAICSHSDFQDIPYSDWCEQTIHNPLEVVPSKFSGISGCSDGVSQEGSASSTKSTELLLGVKT
IPDDTPMCRILLRKEVLRLVINLSSSVSTKCHETGLLTIKEKYPQTFDDICLYSEVSHLLSHCTFRLPCRRFIQELFQDV
QFLQMHEEAEAVLATPPKQPIVDTSAES.

Figure 8

GβL mRNA sequence (i.e. includes UTRs and ORF) **(accession # BC017119)**

GTGCGGAGCCGCCCGTAAGATGCTCTGACCTTTGACCCTGCCGTTCAGCTCTAGGGCCCGTGCAGGCCACACCATGAACA
CCTCCCCAGGCACGGTGGGCAGTGACCCGGTCATCCTGGCCACTGCAGGCTACGACCACACCGTGCGCTTCTGGCAGGCC
CACAGCGGCATCTGCACCCGGACGGTGCAGCACCAGGACTCCCAGGTGAATGCCTTGGAGGTCACACCGGACCGCAGCAT
GATTGCTGCTGCAGGTTACCAGCACATCCGCATGTATGATCTCAACTCCAATAACCCTAACCCCATCATCAGCTACGACG
GCGTCAACAAGAACATCGCGTCTGTGGGCTTCCACGAAGACGGCCGCTGGATGTACACGGGCGGCGAGGACTGCACAGCC
AGGATCTGGGACCTCAGGTCCCGGAACCTGCAGTGCCAGCGGATCTTCCAGGTGAACGCACCCATTAACTGCGTGTGCCT
GCACCCGAACCAGGCAGAGCTCATCGTGGGTGACCAGAGCGGGGCTATCCACATCTGGGACTTGAAAACAGACCACAACG
AGCAGCTGATCCCTGAGCCCGAGGTCTCCATCACGTCCGCCCACATCGATCCCGACGCCAGCTACATGGCAGCTGTCAAT
AGCACCGGAAACTGCTATGTCTGGAATCTGACGGGGGGCATTGGTGACGAGGTGACCCAGCTCATCCCCAAGACTAAGAT
CCCTGCCCACACGCGCTACGCCCTGCAGTGTCGCTTCAGCCCCGACTCCACGCTCCTCGCCACCTGCTCGGCTGATCAGA
CGTGCAAGATCTGGAGGACGTCCAACTTCTCCCTGATGACGGAGCTGAGCATCAAGAGCGGCAACCCCGGGGAGTCCTCC
CGCGGCTGGATGTGGGGCTGCGCCTTCTCGGGGGACTCCCAGTACATCGTCACTGGTGAGCCCCGCCCTGGCCTCCCCCA
TCCCTGGCCCCCGGCGCTGGCCTCCAGAGCCAGCCCACCTCGGCTGCAGCTTCCCCTCTGCTGGGGCCGCCTGCTTGGCC
TGCACCTGCGCTCTTAGCCCTGCACAATCTCCCCCTCCAGCTTCCTCGGACAACCTGGCCCGGCTCTGGTGTGTGGAGAC
TGGAGAGATCAAGAGAGAGTATGGCGGCCACCAGAAGGCTGTTGTCTGCCTGGCCTTCAATGACAGTGTGCTGGGCTAGC
CTGTGACCCCTCGGGACTGCCTGGTGCAGGTGGTGGCAGCTGGAGGGACCCATGCAGCACCCAGGTCAGAGCAGACCCTC
CCCTGCCGGCCTGCGCCAGCTGGACCTGATGGCCCCCTGTGGCGCCTTGACCTGCTGGGCCAGGCTGCCCTGGGACTCTC
AGCCCCCAGTTGCTTATCCAGATGTGACAGAGCTCGACCCAAGCCAGGCTGCACACTCCTGGACTGGGCTAGCCTGCACT
GCCTGGGAAAGTCGGCCGAGGGCCCAAAGCTGCTGAGGGGTCTGAGGCTGGTGCCCACCCCCAAGCTAGTGCGTTCTCTG
CCCCTCCCTGCCCGCGTTTCAGGGCCTCGGTCCATAGAGAACACCACCACCATGGCCAGGTGGAAGGGTTTATTAGTCCC
TGCCAGCAGCTGTCCTCCCTGGTGCAGGTGGCCTGGCCAGCCCACTGGATTGGGGACGGGCCAGGCTGGGCCAGGTCGGG
GGCTCAGTCTGGGAGGTAATAAAAGCAGACCGACACGCAGATGTTGCTCGGGAAAAAAAAAAAAAAAAAAAAAAACCA
AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

Figure 9a

<u>GβL ORF sequence (i.e., no UTRs)</u> **(derived from accession # BC017119)**

ATGAACACCTCCCCAGGCACGGTGGGCAGTGACCCGGTCATCCTGGCCACTGCAGGCTACGACCACACCGTGCGCTTCTG
GCAGGCCCACAGCGGCATCTGCACCCGGACGGTGCAGCACCAGGACTCCCAGGTGAATGCCTTGGAGGTCACACCGGACC
GCAGCATGATTGCTGCTGCAGGTTACCAGCACATCCGCATGTATGATCTCAACTCCAATAACCCTAACCCCATCATCAGC

Figure 9b

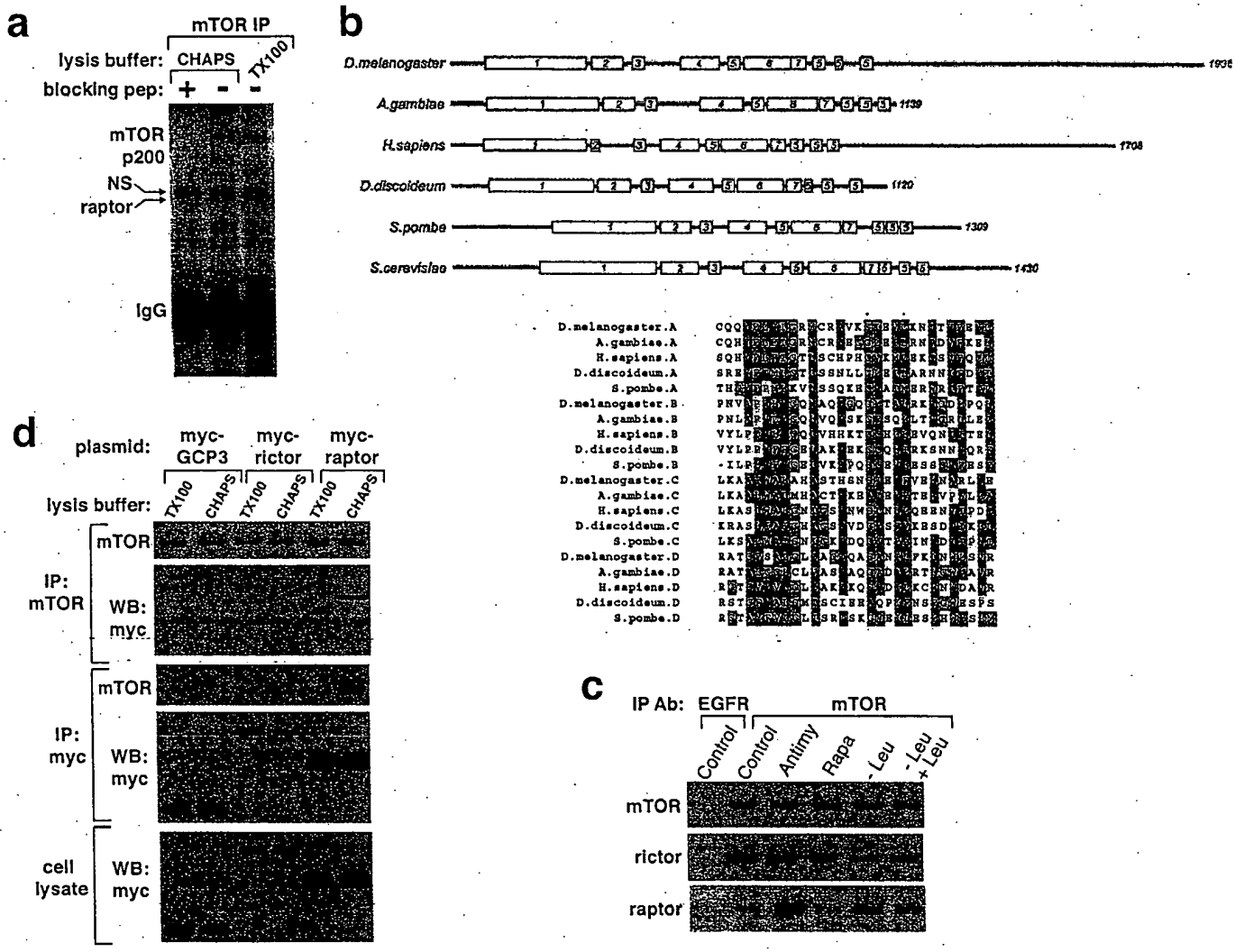
TACGACGGCGTCAACAAGAACATCGCGTCTGTGGGCTTCCACGAAGACGGCCGCTGGATGTACACGGGCGGCGAGGACTG
CACAGCCAGGATCTGGGACCTCAGGTCCCGGAACCTGCAGTGCCAGCGGATCTTCCAGGTGAACGCACCCATTAACTGCG
TGTGCCTGCACCCGAACCAGGCAGAGCTCATCGTGGGTGACCAGAGCGGGGCTATCCACATCTGGGACTTGAAAACAGAC
CACAACGAGCAGCTGATCCCTGAGCCCGAGGTCTCCATCACGTCCGCCCACATCGATCCCGACGCCAGCTACATGGCAGC
TGTCAATAGCACCGGAAACTGCTATGTCTGGAATCTGACGGGGGGCATTGGTGACGAGGTGACCCAGCTCATCCCCAAGA
CTAAGATCCCTGCCCACACGCGCTACGCCCTGCAGTGTCGCTTCAGCCCCGACTCCACGCTCCTCGCCACCTGCTCGGCT
GATCAGACGTGCAAGATCTGGAGGACGTCCAACTTCTCCCTGATGACGGAGCTGAGCATCAAGAGCGGCAACCCCGGGGA
GTCCTCCCGCGGCTGGATGTGGGGCTGCGCCTTCTCGGGGGACTCCCAGTACATCGTCACTGCTTCCTCGGACAACCTGG
CCCGGCTCTGGTGTGTGGAGACTGGAGAGATCAAGAGAGAGTATGGCGGCCACCAGAAGGCTGTTGTCTGCCTGGCCTTC
AATGACAGTGTGCTGGGCTAG

Figure 10

GβL amino acid sequence **(accession # AAH17119)**

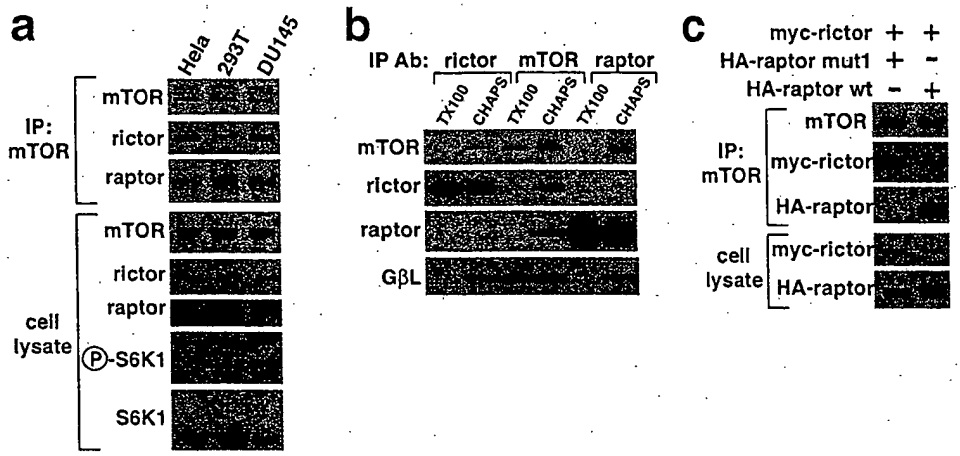
MNTSPGTVGSDPVILATAGYDHTVRFWQAHSGICTRTVQHQDSQVNALEVTPDRSMIAAAGYQHIRMYDLNSNNPNPIIS
YDGVNKNIASVGFHEDGRWMYTGGEDCTARIWDLRSRNLQCQRIFQVNAPINCVCLHPNQAELIVGDQSGAIHIWDLKTD
HNEQLIPEPEVSITSAHIDPDASYMAAVNSTGNCYVWNLTGGIGDEVTQLIPKTKIPAHTRYALQCRFSPDSTLLATCSA
DQTCKIWRTSNFSLMTELSIKSGNPGESSRGWMWGCAFSGDSQYIVTGEPRPGLPHPWPPALASRASPPRLQLPLCWGRL
LGLHLRS.

Figure 11



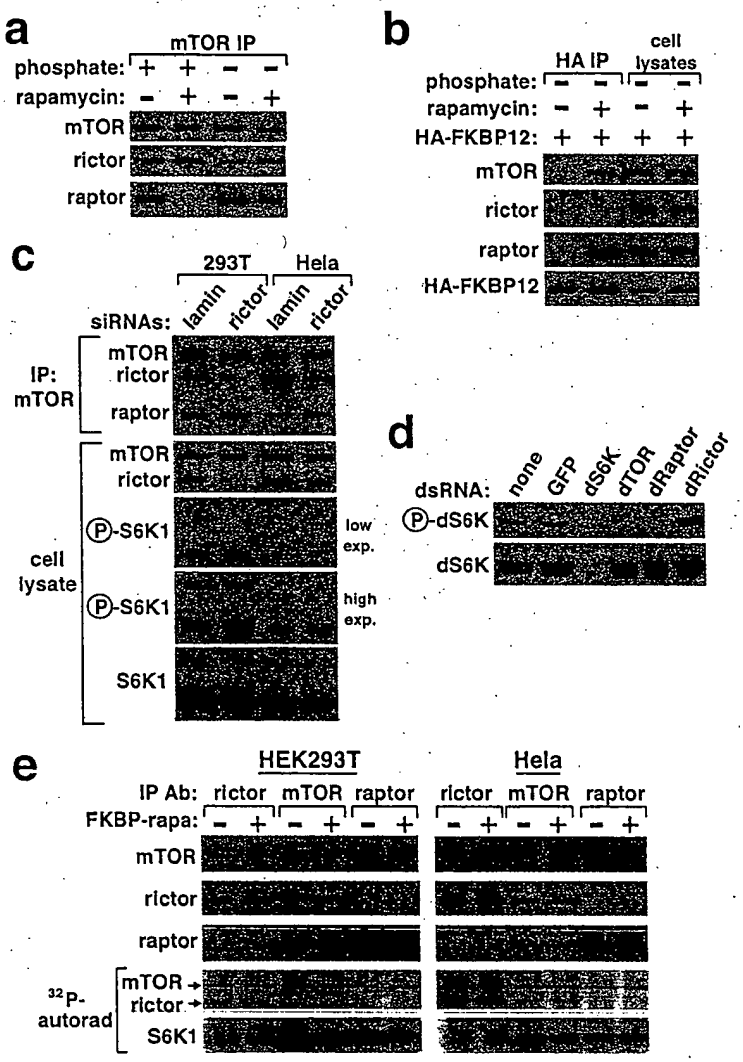
Rictor is a novel mTOR-associated protein. a, Silver stain of SDS-PAGE analysis of mTOR immunoprecipitates prepared from HeLa cells lysed in a CHAPS- or Triton X-100-containing buffer. (+) indicates inclusion of the blocking peptide for the mTOR antibody during the immunoprecipitation. The ~200 kDa band corresponds to rictor and a non-specific band (NS) obscures raptor. **b,** Rictor homologues share common domain architectures. Analyses of indicated rictor homologues identified seven domains with sequence conservation and similar relative locations within each protein and are shown schematically as boxes. Domain five is repeated four times within each of the homologues and the multiple sequence alignment shows the sequence pattern of this repeat. **c,** Specific interaction between endogenous mTOR and rictor. Immunoprecipitates prepared with the indicated antibodies were analyzed by immunoblotting for mTOR, rictor and raptor. Prior to use cells were treated with 5 μM Antimycin A for 15 min (Antimy), 20 nM rapamycin for 15 min (Rapa), deprived of leucine for 90 min (-Leu), or deprived of leucine and stimulated with 52 μg/ml leucine for 10 min (-Leu+Leu). **d,** Endogenous mTOR interacts with recombinant rictor and raptor. Cellular lysates and mTOR immunoprecipitates prepared from HEK293T cells expressing myc-rictor, myc-raptor, or myc-GCP3 were analyzed by immunoblotting for myc-tagged proteins. In parallel, anti-myc immunoprecipitates were analyzed by immunoblotting for mTOR.

Figure 12



Rictor and raptor define two distinct mTOR-containing complexes. a, Immunoblot analyses for indicated proteins of mTOR immunoprecipitates and cell lysates prepared from HeLa, HEK293T, and DU145 cells. Equal amounts of total protein were analyzed from each cell type. **b,** Immunoblot analyses for the presence of the indicated components of the mTOR signaling complex in immunoprecipitates prepared from HEK293T cell lysates with antibodies against rictor, mTOR, or raptor. **c,** Recombinant wild type raptor but not a mutant raptor suppresses the binding of rictor to mTOR. mTOR immunoprecipitates prepared from HEK293T cells expressing the indicated tagged proteins were analyzed by immunoblotting with anti-myc and anti-HA antibodies.

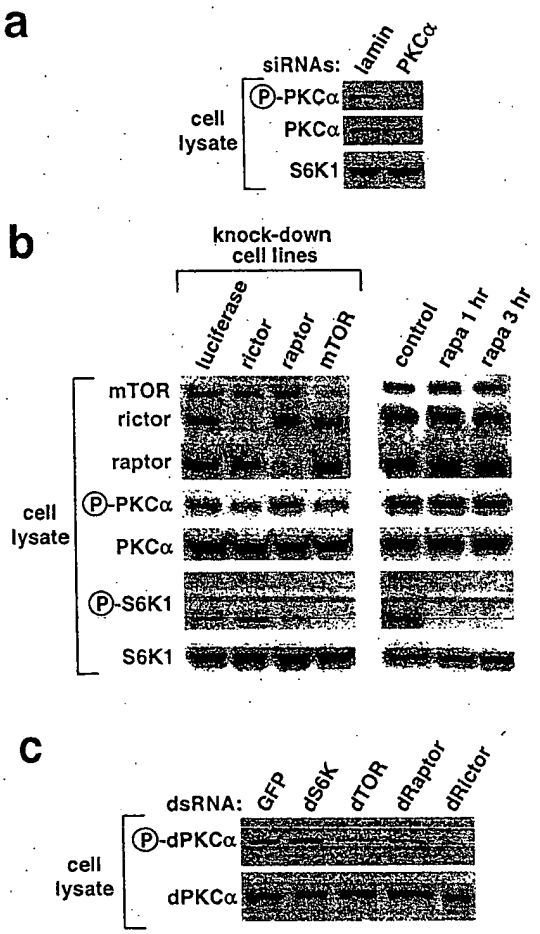
Figure 13



Rictor does not participate in rapamycin-sensitive mTOR functions.

a, The sensitivity of the raptor-mTOR interaction to rapamycin depends on the presence of phosphate-containing molecules in the lysis buffer. mTOR immunoprecipitates prepared from cells treated with or without 20 nM rapamycin for 10 min and lysed in a phosphate-containing or phosphate-free buffer were analyzed by immunoblotting for the indicated proteins. **b,** Raptor and mTOR, but not rictor, co-purify with FKBP12-rapamycin. Anti-HA immunoprecipitates prepared from HEK293T cells expressing HA-FKBP12 and treated with or without 20 nM rapamycin for 15 min were analyzed by immunoblotting for the indicated proteins. **c,** Suppression of rictor expression slightly increases the amount of raptor in the mTOR complex and S6K1 activity. mTOR immunoprecipitates and cell lysates prepared from HEK293T or HeLa cells transfected with siRNAs targeting lamin or rictor were analyzed by immunoblotting for the indicated proteins. **d,** Suppression of Drosophila rictor expression increases the phosphorylation state of dS6K. The indicated dsRNAs were applied to Drosophila S2 cells and cell lysates were analyzed by immunoblotting with the mammalian phospho-specific S6K1 and Drosophila S6K antibodies. **e,** The rictor-containing mTOR complex does not phosphorylate S6K1. Immunoprecipitates prepared with the indicated antibodies were used in mTOR kinase assays using S6K1 as a substrate[1]. Where indicated immunoprecipitates were treated with 100 nM FKBP12-rapamycin for 40 min before the start of the assays. Immunoblotting was used to monitor the levels of rictor, mTOR, and raptor in the kinase reactions.

Figure 14



Rictor and mTOR, but not raptor, regulate the PKCα phosphorylation state in human and Drosophila cells. a, siRNA-mediated reduction in the expression of total PKCα in HeLa cells also reduces the immunoblot signal from a phosphospecific antibody recognizing phospho-S657 of PKCα but does not affect the levels of S6K1. **b,** Immunoblotting was used to analyze the phosphorylation states of PKCα and S6K1 in HeLa cells with reduced expression of rictor, raptor, or mTOR or treated with rapamycin. Lentiviruses were used to express siRNAs targeting rictor, raptor, mTOR or luciferase. **c,** dsRNAs corresponding to the genes for the indicated proteins were applied to S2 Drosophila cells. After 4 days lysates were prepared and analyzed by immunoblotting for dPKCα and phospho-dPKCα levels.

Figure 15ab

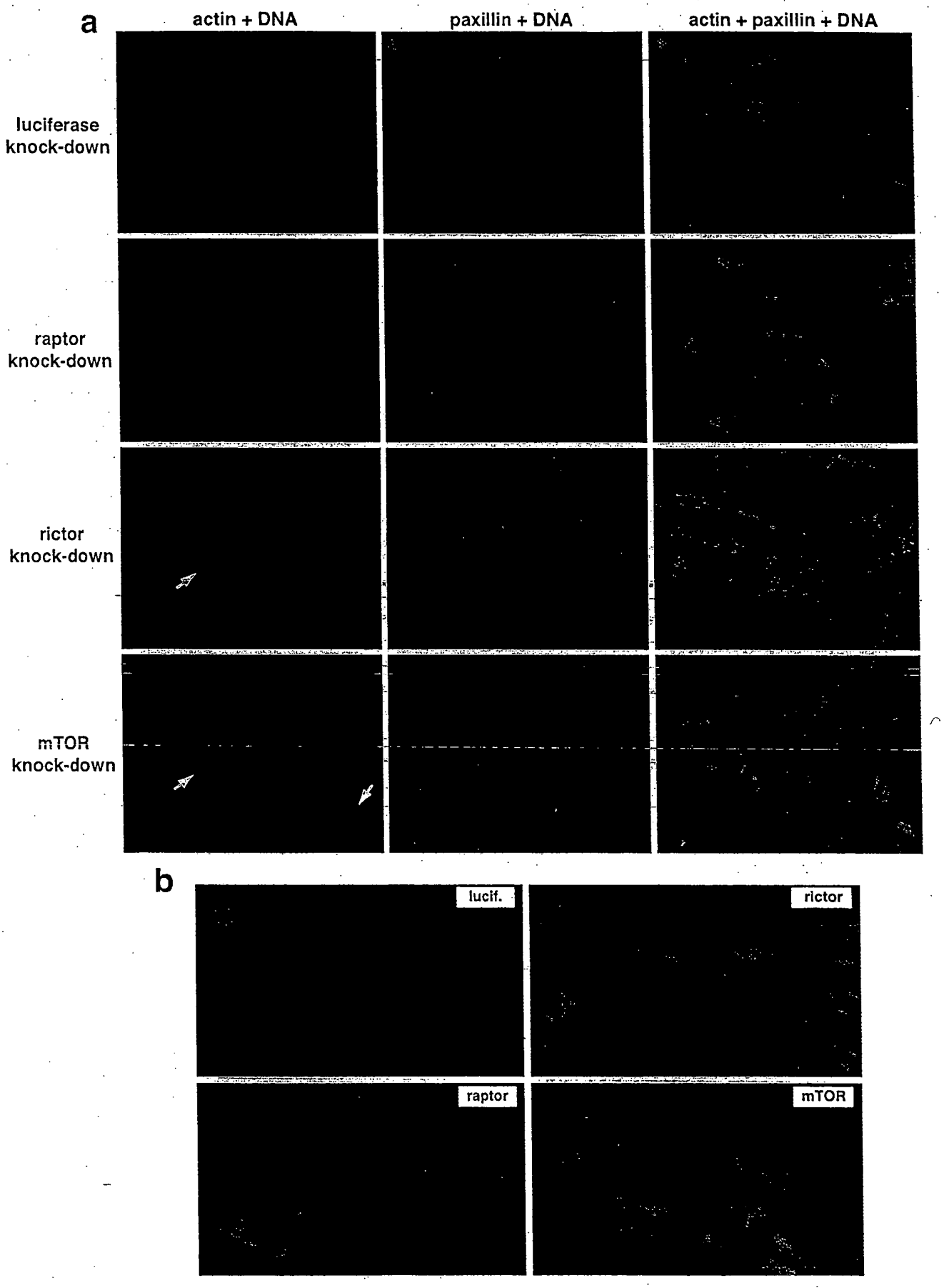
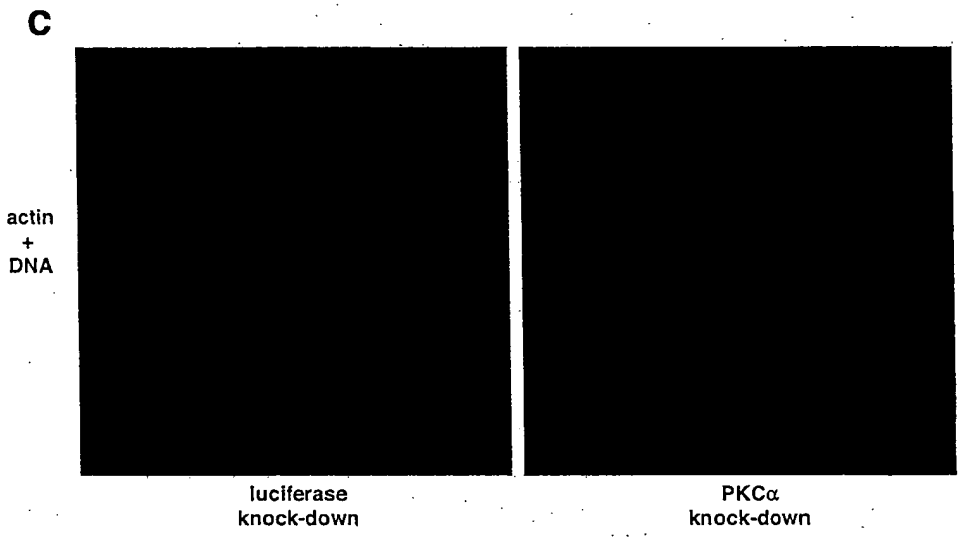
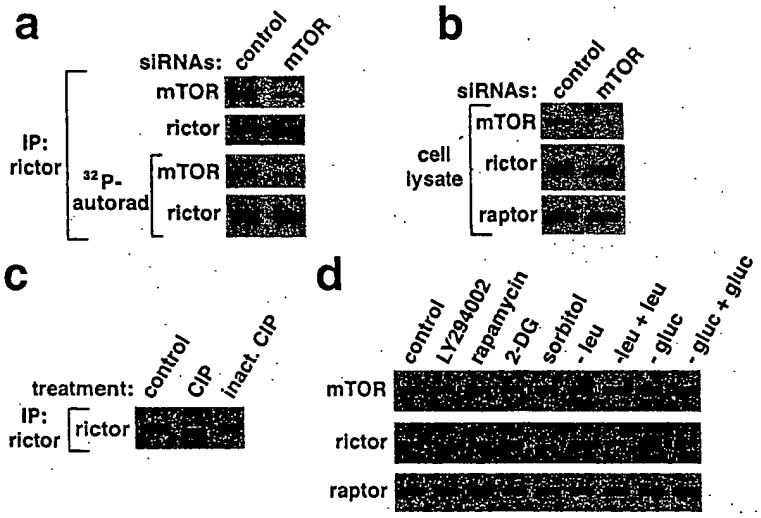

a
actin + DNA
paxillin + DNA
actin + paxillin + DNA
luciferase knock-down
raptor knock-down
rictor knock-down
mTOR knock-down
b
lucif.
rictor
raptor
mTOR

Figure 15c



Rictor, mTOR and PKCα regulate the organization of the actin cytoskeleton. a, Staining for actin (red), paxillin (green) and DNA (blue) reveals the organization of the actin cytoskeleton in HeLa cells transduced with the siRNA-expressing lentiviruses described in Figure 4a. Arrows point to bundles of actin fibers. Images captured with a 60x objective are shown. **b,** Higher magnification of portions of the merged images from Figure 5a. **c,** Like cells with reduced rictor expression, cells with reduced expression of PKCα have an altered actin cytoskeleton.

Figure 16



mTOR regulates the rictor phosphorylation state. a, HeLa cells with reduced expression of mTOR or of a control protein were metabolically labeled with ^{32}P and the level of phosphorylated rictor determined by immunoprecipitation followed by autoradiography and immunoblotting for the indicated proteins. **b,** The mobility of rictor in SDS-PAGE is affected by mTOR. HeLa cells with siRNA-mediated reductions in mTOR or controls were analyzed by immunoblotting for mTOR, rictor, and raptor. **c,** The phosphorylation state of rictor affects its mobility in SDS-PAGE. Rictor immunoprecipitates were incubated with or without calf intestinal phosphatase (CIP) or heat inactivated CIP and analyzed by SDS-PAGE and immunoblotting for rictor. **d,** Osmotic stress increases the mobility of rictor in SDS-PAGE. Lysates of HeLa cells exposed for 1 hr to 20 nM rapamycin, 20 nM LY294002, 100 mM 2 deoxyglucose (2-DG), medium without leucine or glucose, or medium without leucine or glucose followed by the readdition of the missing component for 10 minutes were analyzed by immunoblotting for rictor.

Figure 17

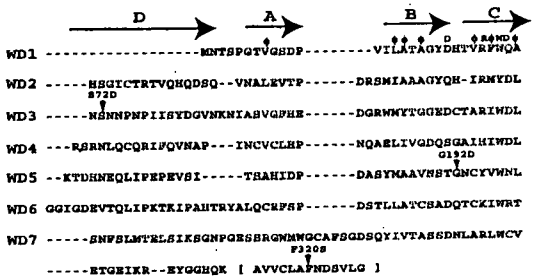


Figure 18

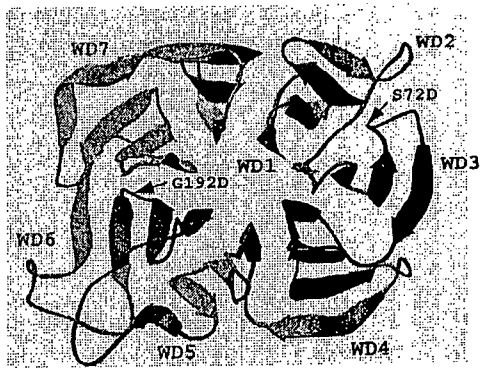


Figure 19